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THE EFFECTS OF SELECTED AQUATIC SEDIMENTS ON THE ACUTE TOXICITY OF N-NITROSODIMETHYLAMINE TO GAMMARUS LIMNAEUS

A. C. DRAPER III
J. W. FISHER

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AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

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AMRL-TR-79-94

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals, "Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

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This study was designed to provide information concerning the acute toxicity effects of N-nitrosodimethylamine to Gammarus limnaeus in the presence of two types of aquatic sediments. Analytical extraction techniques for the recovery of the N-nitrosodimethylamine from these sediments were developed. Recovery efficiencies of 69.6% and 91.3% were achieved. The nature of the sediments had a definite effect on the 96h continuous-flow LC_{50} value.

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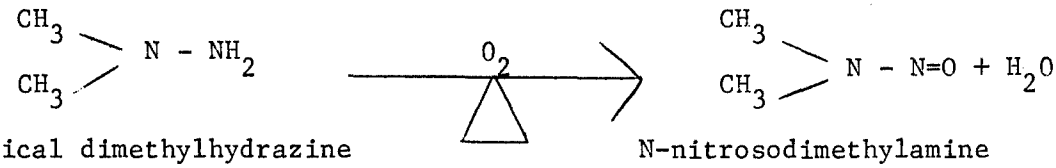
PREFACE

This study was conducted in the Toxic Hazards Division, Environmental Quality Branch, Aerospace Medical Research Laboratory. The research was performed in support of Project 6302, "Occupational and Environmental Toxic Hazards in Air Force Operations", Task 04, Workunit 18, from September 1978 to April 1979.

The authors wish to thank Dr. W.S. Brewer, Wright State University, for his suggested analytical techniques, Dr. Roger Inman, Toxicology Branch, for his suggested statistical analyses, Dr. Melvin Andersen, Toxicology Branch, for reviewing this manuscript, Dave Geiger and other Environmental Quality Branch personnel supporting this study.

INTRODUCTION

Volatile nitrosamines represent a group of compounds that are known to be highly carcinogenic, mutagenic, and teratogenic (Magee and Barnes, 1967; Magee and Swann, 1969; and Druckery and Landschwetz, 1971). Not much is known about the acute toxicity of these compounds in the aquatic environment. Nitrosamines have been detected as contaminants in hydrazine rocket fuels (Heck et al., 1962) and are oxidative products of such compounds (Serfontain and Hurter, 1966; Figure 1).



Unsymmetrical dimethylhydrazine N-nitrosodimethylamine

Figure 1. Oxidation of UDMH to N-nitrosodimethylamine.

Hydrazine's toxic properties have been assessed with LC_{50} values of 0.61 mg/ℓ to 26.5 mg/ℓ being reported for the common guppy depending on the type of hydrazine used and the hardness of the water (Slonim, 1977). Fisher et al. (1978) reported an LC_{50} value of 1.08 mg/ℓ for bluegills with hydrazine. Scherfig (1977) assessed hydrazine toxicity to algae by observing a decrease in cell number at a concentration as low as 0.05 mg/ℓ.

While nitrosamines have been detected in foods, cosmetics, and pesticides (Sen et al., 1973 and Ayabana et al., 1973), their presence in the aquatic environment has only recently been discovered. Nitrosamines have been detected in sewage sludges effluents (Ayabana, 1974 and Fine et al., 1975) which dump into receiving streams and recently have been detected in municipal sewage sludges which are being applied to agricultural lands (Brewer et al., 1980). Since nitrosamines placed in soils have been shown to leach through the soils quite readily, the potential for ground water contaminants exists (Dean-Raymond and Alexander, 1976). Once in soils and water, nitrosamines are relatively resistant to biodegradation (Tate and Alexander, 1975). Proteus mirabilis has been shown to release N-nitrosodimethylamine as a degradation product (Brooks et al., 1972). N-nitrosodimethylamine has been detected along with some of its derivatives in drinking water in the low ppb range (Fine et al., 1977).

Due to the dynamic and complex chemical and microbiological interactions that may occur between sediments and toxicants in aqueous solution standard bioassay procedures have not included sediments. However, it is difficult to identify an aquatic environment without some type of sediment associated with it. The present study was carried out to determine the effects different sediments have upon the acute toxicity of N-nitrosodimethylamine to Gammarus limneaus, an aquatic invertebrate. This type of study required that the sediment be analyzed for nitrosamine concentration (exclusion or accumulation). Therefore, analytical extraction

procedures were developed for the recovery of N-nitrosodimethylamine from the sediment.

MATERIALS AND METHODS

Continuous-flow System: A continuous-flow bioassay test system was constructed using five channel multiflex peristaltic pump, 10 liter toxicant holding tanks and two liter exposure chambers. All connections were made with either glass or tygon (Figure 2). Pollutant monitoring points were made by hooking intravenous 16 gage "Butterflys" at the test chamber points. This permitted monitoring of exact concentrations delivered into each test chamber, thereby correcting for any photodecomposition, chemical decomposition, plating or other type chemical loss which may have taken place between holding tanks and the test chambers. Disposal of the N-nitrosodimethylamine effluent was carried out by oxidation with a 10% hypochlorite solution[1] (MacNaughton, personal communication).

Sediment Analysis: Sediment samples were taken from Bass Lake (WPAFB Dayton, Ohio) and a small eutrophic farm pond about 5 miles north of Xenia, Ohio. After retrieving the samples, the top five cm of sediment were collected and 200 grams were placed in each two liter test chamber (Figure 2), then were exposed to 0.1 mg/ℓ, 1.0 mg/ℓ, and 10.0 mg/ℓ of N-nitrosodimethylamine and a control for a period of 96 hrs. After each test period samples were filtered through coarse filter paper to remove excess water. The remaining wet solids plus an added 3.0 mℓ distilled water were placed into preweighed extraction thimbles. The thimbles were then placed into Soxhlet extractors containing methylene chloride (100 mℓ) for three hours. The samples were then evaporated slowly in an evaporative concentrator at 30°C (in vacuo) until all methylene chloride had evaporated. The remaining sample (5-8 mℓ) was passed through XAD-2 resin which had been pretreated by passing three 25 mℓ aliquots of acetone, methanol, and distilled water through the column. This acted as an organic clean-up procedure and only contributed a maximum of 4% loss in recovery. The column was then washed with 2-5 mℓ of distilled water (final sample volume 10 mℓ).

Aliquots of the aqueous samples were directly injected onto a 0.3 cm x 3.0 m glass column packed with 80/100 mesh Chromosorb 103 AW (18). Operating parameters of the Varian Model 2100 gas chromatograph were: column temperature, 180°C, injection temperature, 220°C, detector temperature, 240°C, nitrogen flow rate, 60 mℓ/min and injected volume, 1-3 μℓ. Quantitation was accomplished by comparing areas of sample peaks to authentic N-nitrosodimethylamine standards (Aldrich Chemical Company).

[1] Mike MacNaughton, Major, BSC, CEEDO, Tyndall Air Force Base, Florida, personal communication.

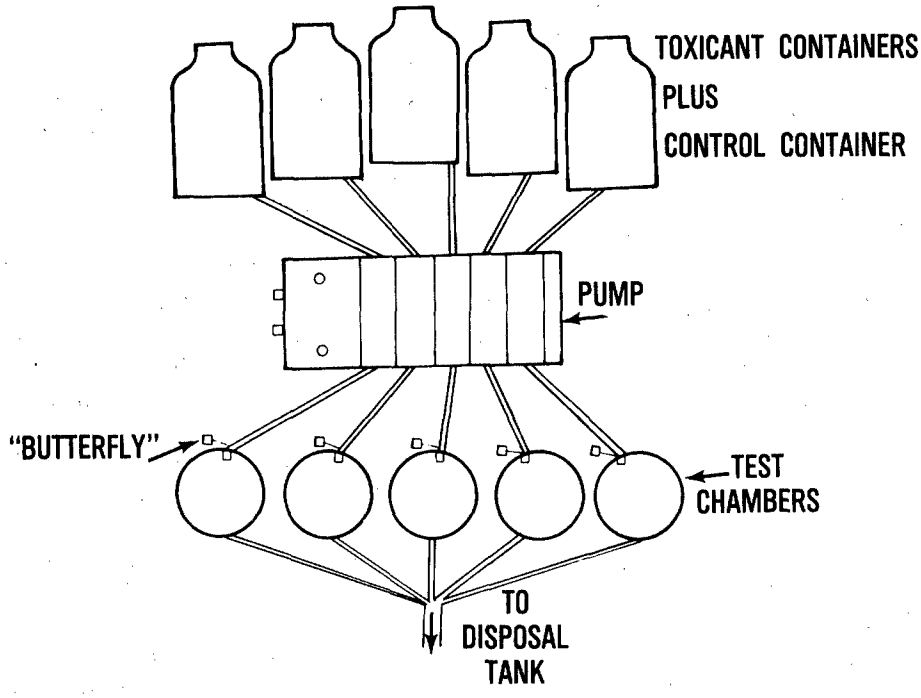


Figure 2. Schematic of experimental design.

Invertebrate Bioassay: Gammarus limnaeus, an amphipod commonly referred to as a side swimmer or a scud, was used as the test organism. This organism, a widely distributed aquatic invertebrate in midwestern lakes and streams (Figure 3), was chosen due to its large size (3-5 mm), wide distribution, ability to burrow in sediment, and ease in handling (Pennak, 1953).

Twenty organisms per test chamber were first exposed to concentrations of N-nitrosodimethylamine ranging from 100 mg/ℓ to 500 mg/ℓ in the continuous-flow system with controls (Figure 2). A 96h continuous-flow LC_{50} without sediment was determined. Another 96h continuous-flow bioassay with 20 organisms per test chamber was exposed to a concentration range of 50 mg/ℓ to 600 mg/ℓ of N-nitrosodimethylamine in the continuous-flow system; however, 200 grams of Bass Lake sediment was included in each test chamber. A similar experiment was conducted using 200 grams of Farm Pond sediment. Nitrosamine concentrations were monitored every 24 hours to assess compound loss (Table 1). Initial nitrosamine concentrations were monitored directly from the holding flasks, while subsequent concentrations during the experiments were taken from the "butterfly" in-line connection at the test chamber solution delivery point (Figure 2).

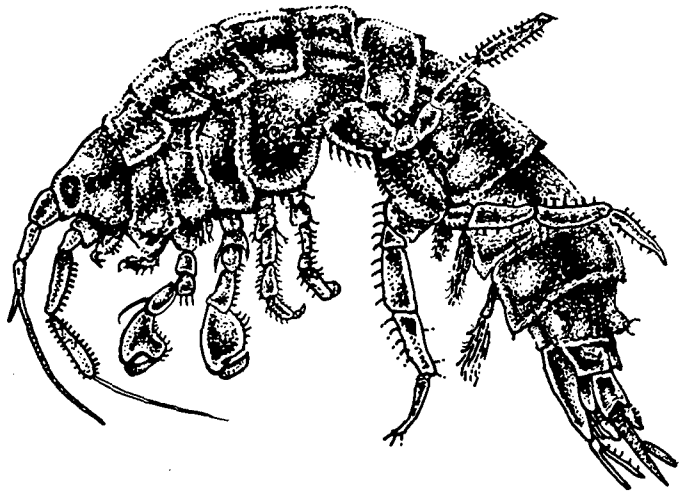


Figure 3. Gammarus limnaeus.

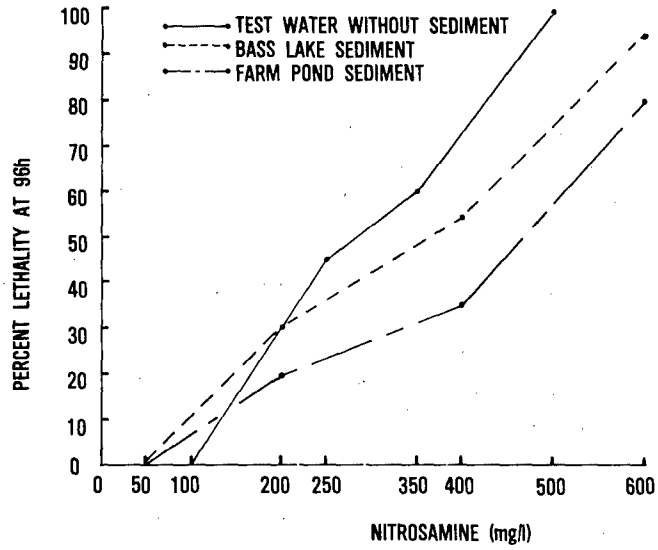


Figure 4. Graphic plot of percent mortality at 96h.

TABLE 1

AVERAGE N-NITROSODIMETHYLAMINE CONCENTRATIONS FROM TOXICANT CONTAINERS DURING BIOASSAYS.

Initial Concentration mg/	0 h mg/ℓ	24 h mg/ℓ	48 h mg/ℓ	72 h mg/ℓ	96 h mg/ℓ
50	50	50	48	48	48
100	100	96	96	92	90
200	190	188	184	181	174
250	235	231	220	212	210
350	330	318	290	278	275
400	384	360	356	342	330
500	480	473	448	433	405
600	573	538	507	479	460

RESULTS

Sediment Analysis: There was very little retention of N-nitrosodimethylamine by either the Bass Lake or the Farm Pond sediments (Table 2). At 10 ppm nitrosamine, recovery percentages of 13.9% (1.39 ppm) and 25.6% (2.56 ppm) for Bass Lake and the Farm Pond sediments, respectively, were achieved. Recovery percentages from known spiked samples were 91.3% and 69.6% (Table 2) for Bass Lake and Farm Pond sediments, respectively. Consequently, it would appear that the levels of N-nitrosodimethylamine retained in the sediment are much less than those in the aqueous phase.

In the 0.1 ppm and 1.0 ppm concentration levels of retention were below detection (Table 2). If these concentrations maintained similar sediment retention values (13.9% and 25.6%), the levels would indeed be below the limits of gas chromatographic detection.

Invertebrate Bioassay: From the dose-mortality curves median lethal concentrations (LC_{50}) and 95% confidence intervals were determined by the Litchfield-Wilcoxon method (1949). A 96h LC_{50} of 280 mg/ℓ (95% C. I. of 254 mg/ℓ - 336 mg/ℓ) was calculated when G. limnaeus was exposed to N-nitrosodimethylamine in the presence of Bass Lake sediment; and a 96h LC_{50} of 445 mg/ℓ (95% C.I. of 342 mg/ℓ - 549 mg/ℓ) was calculated when Gammarus limnaeus was exposed to N-nitrosodimethylamine in the presence of Farm Pond sediment.

TABLE 2

N-NITROSODIMETHYLAMINE RECOVERY FROM SEDIMENTS.

Concentration	Bass Lake Sediment*	Farm Pond Sediment**
0 ppm	N.D.**	N.D.**
0.1 ppm	N.D.	N.D.
1.0 ppm	N.D.	N.D.
10.0 ppm	1.39 ppm	2.56 ppm
Spiked Sample 1 µg/g	0.913 µg/g 91.3% recovery	0.696 µg/g 69.6% recovery

* Value reported is the average of three replicate flasks.

** Not detectable or below the limits of detection.

TABLE 3

CHEMICAL ANALYSIS OF SEDIMENT.

Soil Composition	Bass Lake Sediment	Farm Pond Sediment
Volatile residue *	3.24%	7.83%
Si**	10%	10%
Mg	1%	1%
Al	1%	10%
Fe	1,000 ppm	1%
Ti	100 ppm	1,000 ppm
Na	10,000 ppm	10,000 ppm
K	10,000 ppm	10,000 ppm
Pb	+	10 ppm
Ni	10 ppm	10 ppm
Cu	10 ppm	100 ppm
Zn	10 ppm	10 ppm
Mn	100 ppm	100 ppm
Cr	10 ppm	10 ppm
Ag	+	+
Co	+	+

* Volatile residue includes organic matter and volatile inorganic matter.

** All cations measured by emission spectrophotometry.

\+ Not detected.

DISCUSSION

The invertebrate bioassay test chamber developed allows for the continuous delivery of the pollutant and fresh water, reducing problems associated with the build-up of wastes and nutrient depletion. The system can be run as a closed system to eliminate evaporation loss associated with the testing of highly volatile compounds such as N-nitrosodimethylamine (MacNaughton, 1975). The loss of nitrosamine (Table 1) from the system would therefore appear to be mainly due to photodecomposition (MacNaughton, 1975). The average loss of nitrosamine over a 96h test period ranged from 15.5% to 23%. This would indicate that the LC_{50}s determined are actually an underestimation of actual acute toxicity values.

Percent recoveries associated with the sedimentary extraction procedures were reported as 91.3% and 69.6% for Bass Lake and Farm Pond sediment, respectively. The difference in percent recoveries (21.7%) may be attributed to the higher volatile residue in the Farm Pond sediments (7.83%) as compared to that found in the Bass Lake sediment (3.24%) (Table 3). Organic carbon has a high adsorption efficiency associated with it, and therefore would affect the amount of nitrosamine bound in the sediment (Manaham, 1975). Also, greater amounts of Al, Fe, Ti, Pb, and Cu were found in the Farm Pond (Table 3). Nitrosamine may be bound to humic or folic acids, be chelated with heavy metals, or have chemical transformation which would make extraction and identification difficult (Manaham, 1975). Binding should reduce the toxicity or availability of the nitrosamine to the organism tested.

Effect of sediment on N-nitrosodimethylamine toxicity to Gammarus limnaeus was quite clear. There was a marked difference between the standard LC_{50} value without sediment (280 mg/ℓ) and the LC_{50} value with the addition of the Farm Pond sediment (445 mg/ℓ). Although the LC_{50} value with the addition of Bass Lake sediment was greater (310 mg/ℓ), the 95% confidence intervals overlapped the standard LC_{50} 95% confidence intervals. This indicates that no statistical differences between these LC_{50} values exist. Differences in LC_{50} values between sediments vs. non-sediment bioassays may be attributed to the ability of the organism to burrow into the sediment, thus escaping from the higher concentrations associated with the aqueous phase.

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Transfer of ^{14}C-Toluene from Mosquito Larvae to Bluegill Sunfish

W. O. Berry* and J. W. Fisher

Environmental Quality Branch, Toxic Hazards Division, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio 45433

Toluene is one of the main water-soluble fractions of refined petroleum products such as gasoline (BERRY and BRAMMER 1977, BERRY and STEIN 1977) and jet fuels (R. L. PUYEAR, personal communication). Previous studies have demonstrated the uptake of toluene from aqueous solutions by mosquito larvae (BERRY et al. 1978) and bluegill sunfish (BERRY 1979). The transfer of toluene from fourth-instar mosquito larvae to bluegills feeding on such larvae is reported in this paper.

EXPERIMENTAL

Toluene-water solutions were prepared by adding 0.5 mℓ of ^{14}C-toluene (S.A. = 4mCi/mmol) to one liter of water in a one liter flask. This mixture was then stirred for 3 hrs in a stoppered flask, placed in a separatory funnel and permitted to separate for 20 hrs. The lower 950 mℓ of this solution was removed to another flask and stirred for 10 minutes. This mixture was transferred to a 1.5 liter glass bowl and 500 fourth-instar larvae of the mosquito Aedes aegypti were added to the solution and permitted to incorporate radioactive toluene for 3 hrs. At the end of the 3 hr exposure period, a group of 5 mosquito larvae were added to each of 5 scintillation vials. Tissue solubilizer (1 mℓ Soluene-350) was added to each vial containing 5 larvae and tissue digestion was permitted to proceed for the next 24 hrs at room temperature (22°C). Following digestion, 10 mℓ of scintillation cocktail (Dimilume-30) was added to each vial. Prepared vials were counted on a scintillation spectrometer to determine the amount of ^{14}C-toluene accumulated by the mosquitoes.

Twenty-five bluegill sunfish (mean wt = 6.77 gm) were placed individually in separate 500 mℓ beakers of

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*Present Address: Directorate of Life Sciences
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clean water. Fish were not fed for 48 hrs prior to experimentation. Each fish was given 10 exposed mosquito larvae on which to feed. Although the period of time to consume 10 larvae varied between fish, all fish had eaten 10 larvae within 45 minutes. All 25 fish were then placed in a 15 liter aquarium containing untreated water. At selected time periods (1, 4, 8, 24 and 48 hrs) following their ingestion of ^{14}C-toluene exposed larvae, 5 bluegills were selected at random and sacrificed. The spleen, gall bladder, liver, stomach, gut and kidney were excised from these fish, placed in scintillation vials, weighed, and 1 mℓ of tissue solubilizer added. Following 24 hrs of tissue digestion, 10 mℓ of scintillation cocktail was added to each vial and the amount of ^{14}C-toluene present in each organ was analyzed by scintillation counting. For each time period, a single control fish which had been fed unexposed mosquito larvae was also sacrificed and analyzed for organ concentration of radioactive toluene.

RESULTS and DISCUSSION

The mean uptake of ^{14}C-toluene by fourth-instar mosquito larvae which were fed to bluegill sunfish was 368 ± 42 and 1598 ± 110 counts per minute (cpm) for experiments I and II, respectively. The large difference in the uptake of toluene by mosquito larvae during the two experiments was not found in previous studies (BERRY et al 1978), but can be explained by differences in the amount of ^{14}C-toluene in solution during larval exposures. Radiometric analysis of water samples from ^{14}C-toluene solutions in which the mosquitoes were exposed revealed a 10-fold difference in the amount of labeled toluene in solution between experiments (i.e., 685 cpm and 6946 cpm in experiments I and II, respectively). Values for control larvae were 27-39 cpm for both experiments.

The concentrations of ^{14}C-toluene found in specific bluegill organs for the various times post-feeding on exposed mosquito larvae are shown in Table 1 for experiments I and II. Control values for bluegill organs ranged from 29-40 cpm. Examination of Table 1 demonstrates that, with the exception of the stomach and intestine, there was no difference in the concentration of ^{14}C-toluene in the organs of experimental and control fish. The large accumulations of toluene in the stomach at hours 1, 4 and 8 as well as the modest concentration in the intestine during the intermediate time periods is not surprising. This would be expected as the ingested toluene-treated larvae pass along the alimentary canal. It is obvious that a very insignificant amount

TABLE I

Organ concentration of ^{14}C-toluene from bluegills fed toluene-treated mosquito larvae.

Organ*		Time Post-Feeding (hr) 1	4	8	24	48
Sp	1	30.0 (1.9)#	30.2 (0.3)	30.9 (1.4)	38.5 (4.5)	37.6 (1.9)
	2	36.6 (5.0)	40.0 (4.7)	41.0 (4.1)	39.4 (3.6)	37.4 (4.3)
GB	1	29.6 (2.1)	27.7 (1.9)	30.5 (1.1)	32.5 (4.0)	29.7 (0.8)
	2	31.5 (3.3)	35.5 (2.6)	34.3 (2.9)	38.3 (4.3)	38.0 (2.6)
L	1	34.3 (0.9)	34.9 (3.2)	36.2 (5.9)	42.7 (5.0)	42.7 (7.8)
	2	39.0 (6.2)	42.2 (8.1)	39.4 (9.3)	37.4 (4.3)	34.8 (3.1)
ST	1	102.0 (77.5)	109.1 (76.8)	121.1 (23.4)	66.7 (22.4)	39.5 (4.0)
	2	491.8 (264.3)	338.8 (156.4)	48.2 (16.2)	35.6 (1.8)	32.4 (3.4)
I	1	34.5 (2.4)	36.8 (2.2)	43.6 (7.3)	52.0 (9.3)	35.2 (1.2)
	2	46.6 (12.2)	72.6 (44.5)	55.2 (14.8)	49.2 (7.0)	34.4 (2.5)
K	1	31.6 (1.6)	32.4 (3.3)	32.3 (1.0)	33.8 (3.0)	32.0 (2.4)
	2	34.6 (2.6)	32.8 (3.4)	30.8 (1.9)	33.0 (2.0)	31.0 (3.2)

*Sp=Spleen, GB=Gall Bladder, L=Liver, St=Stomach, I=Intestine, K=Kidney, 1=Experiment I, 2=Experiment II.

#Values are the Mean counts per minute ($\pm$ S.E.) of organs from 5 fish.

of toluene, if any, leaves the digestive tract to be accumulated in other vital organs of the bluegill. This finding is in direct contrast to a previous study which demonstrated that large concentrations of ^{14}C-toluene are found in the spleen, gall bladder, gut and kidney of bluegills exposed to solutions of ^{14}C-toluene containing no food organisms (BERRY 1979). The results reported here support the suggestion of MACEK et al (1977) that bio-accumulation of chemicals within aquatic food chains is insignificant when compared to accumulation directly from water.

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